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Determination of Diphenylamine in Industrial and Municipal Wastewaters

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DETERMINATION OF DIPHENYLAMINE IN INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

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16. ABSTRACT

A method was developed for the determination of diphenylamine in wastewaters. The method development program consisted of a literature review; determination of extraction efficiency for each compound from water using methylene chloride; development of a deactivated silica gel cleanup procedure; and determination of suitable gas chromatographic (GC) analysis conditions.

The final method was applied to Columbus POTW secondary effluent in order to determine the precision and accuracy of the method. The wastewater was spiked with diphenylamine at levels of 5 µg/L and 50 µg/L. Recovery for diphenylamine at the 5 µg/L level was 120 ± 25 percent. Recovery at the 50 µg/L level was 89 ± 11 percent. The method detection limit (MDL) for diphenylamine in distilled water was 1.6 µg/L. In wastewaters it may be higher due to interfering compounds.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid wastes.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.

- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report is one of a series that investigates the analytical behavior of selected pesticides and suggests a suitable test procedure for their measurement in wastewater. The method was modeled after existing EPA methods being specific yet as simplified as possible.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

ABSTRACT

A method was developed for the determination of diphenylamine in wastewaters. The method development program consisted of a literature review; determination of extraction efficiency for each compound from water using methylene chloride; development of a deactivated silica gel cleanup procedure; and determination of suitable gas chromatographic (GC) analysis conditions.

The final method was aplied to Columbus Publicly Owned Treatment Works (POTW) secondary effluent in order to determine the precision and accuracy of the method. The wastewater was spiked with diphenylamine at levels of 5 µg/L and 50 µg/L. Recovery for diphenylamine at the 5 µg/L level was 120 ± 25 percent. Recovery at the 50 µg/L level was 89 ± 11 percent. The method detection limit (MDL) for diphenylamine in distilled water was 1.6 µg/L (7). In wastewaters it may be higher due to interfering compounds.

This report was submitted in partial fulfillment of contract No. 68-03-2956 by Battelle Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from February 1, 1982 to April 30, 1983.

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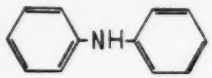
SECTION 1

INTRODUCTION

Diphenylamine(I) is used as a stabilizer in smokeless powders (1,2) as well as to control superficial scald in some varieties of pears and apples (3,4).



I

The CAS registry number for diphenylamine is 122-39-4 and its IUPAC name is N-phenylbenzeneamine. It has a melting point of 53-54°C, a boiling point of 302°C, and an oral LD50 in rats of 300-1000 mg/kg. Common synonyms for diphenylamine include "Anilinobenzene", "DFA", and "DPA". A literature review described extractions of diphenylamine from water with methylene chloride (5) and a cleanup procedure using Bio-Beads S-X2(6). Several columns were used for determination of diphenylamine by GC including 3% SP-1000 (5), 15% UC W-98 and 3% OV-17(2), 3% OV-17 with 0.02% Epikote 1001(4), and 10% OV-101, 6% OV-17, and 5% OV-225(6). Detectors used for GC analyses included a Hall detector (6), a rubidium bead alkali flame detector (AFD) (4,6), and a mass spectrometer (MS) (2,5). Electron-impact (EI) and chemical-ionization (CI) mass spectra were also reported (1,2).

Diphenylamine is stable in water at neutral pH, can be extracted from water with methylene chloride, and contains nitrogen. For these reasons, the selected approach to the determination of diphenylamine in water included extraction from water using continuous extraction with methylene chloride, cleanup using silica gel chromatography, and analysis using packed column GC-AFD. Standard concentration techniques using Kuderna-Danish (K-D) equipment were used. The final method is included in Appendix A of this report.

SECTION 2

CONCLUSIONS

EXTRACTION AND CONCENTRATION

Diphenylamine can be extracted from water using methylene chloride with greater than 90 percent recovery by means of continuous extraction techniques. Use of K-D concentration equipment to perform extract concentrations did not significantly affect compound recoveries.

CLEANUP

Diphenylamine elutes from deactivated silica gel in six percent ethyl ether in petroleum ether with greater than 90 percent recovery. This use of deactivated silica gel was an effective cleanup procedure for extracts from a columbus POTW secondary effluent, but was not assess for extracts from any relevant wastewater samples.

CHROMATOGRAPHY

Two packed GC columns, 3% SP-2250 and 3% SP-1000, were found to be acceptable for the GC-AFD analysis of diphenylamine. The 3% SP-2250 gave better peak shape and was used as the primary column. The 3% SP-1000 column was designated as the alternate column.

VALIDATION STUDIES

Recoveries of diphenylamine from distilled water in the 10 to 1000 µg/L concentration range were greater than 85 percent. Analytical curves constructed from this data were linear. The MDL in distilled water was 1.6 µg/L. Recoveries of diphenylamine from Columbus secondary POTW effluent at the 5 and 50 µg/L levels were 120 ± 25 percent and 89 ± 11 percent, respectively.

SECTION 3

EXPERIMENTAL

Studies were performed to determine if extractions with separatory funnel and continuous extractors, cleanup by silica gel adsorption chromatography, concentration using K-D equipment, and analysis using packed column GC-AFD would be applicable techniques for the determination of diphenylamine in water. Since recovery data and literature references indicated that diphenylamine is relatively stable in water, stability studies were not performed.

EXTRACTION AND CONCENTRATION

Extraction of diphenylamine from water was studied using both separatory funnel and continuous extraction techniques. In both cases one liter of distilled water was used. The sample was adjusted to pH 7 by addition of 6N sodium hydroxide or 6N sulfuric acid. For the separatory funnel studies, the distilled water was spiked with diphenylamine at the 5, 10, 50, 100, 500, and 1000 µg/L level and extracted three times with 60 mL each of methylene chloride. For the continuous extractor studies the water was spiked at the 10 and 100 µg/L levels and extracted with methylene chloride overnight. These studies were done in duplicate. The extracts were dried by passing them through 10 cm of anhydrous granular sodium sulfate, concentrated to one mL and analyzed by GC-AFD.

CLEANUP

Activated silica gel, 20 grams, was stirred with 100 mL of acetone and 1.2 mL of reagent water for 30 minutes. The slurry was transferred to a chromatographic column, and the solvent was allowed to elute and discarded. The column was then sequentially washed with 20 mL of methylene chloride and 30 mL of petroleum ether. Petroleum ether, 50 mL, was suspended over the silica gel. Amounts of either 10 or 100 µg diphenylamine dissolved in 5 mL of methylene chloride was added to the above mentioned petroleum ether. This solvent plus an additional 50 mL of petroleum ether were eluted from the column and collected (F1). Nine additional 25-mL solvent elutions were collected: six percent ethyl ether in petroleum ether (F2); 15 percent ethyl ether in petroleum ether (F3); 50 percent ethyl ether in petroleum ether (F4); ethyl ether (F5); six percent acetone in ethyl ether (F6); 15 Percent acetone in ethyl ether (F7); 50 percent acetone in ethyl ether (F8); acetone (F9); and six percent methanol in acetone (F10). Each fraction was concentrated to approximately 4 mL after addition of 2.5 mL of toluene. The fractions were then transferred to 5-mL volumetric flasks and diluted to volume with toluene.

CHROMATOGRAPHY

Two columns were evaluated for the determination of diphenylamine, 3% SP-2250 on 100/120 mesh Supelcoport and 3% SP-1000 on 100/120 mesh Supelcoport. The 3% SP-2250 column has a maximum temperature limit of 300°C. The 3% SP-1000 column has a maximum temperature limit of only 250° C.

VALIDATION STUDIES

The MDL for diphenylamine was determined by analyzing seven replicate distilled water samples spiked at the 5 µg/L concentration level (7). The sample extracts were cleaned up using the silica gel cleanup procedure prior to analysis. The amounts recovered were determined by external standard calibration and the MDL was calculated from these data.

Distilled water was also spiked in duplicate at the 10, 50, 100, 500, and 1000 µg/L concentration levels and recoveries of the diphenylamine was determined as described earlier. An analytical curve was generated by plotting the amount spiked into the samples versus the amount recovered from the samples.

A relevant wastewater was not available for diphenylamine and Columbus POTW secondary effluent was used for wastewater validation studies. Seven replicates of the wastewater were analyzed to determine the background levels. The wastewater was spiked with diphenylamine at the 5 and 50 µg/L concentration levels, processed and analyzed. Seven replicate extractions were performed at each concentration level.

SECTION 4

RESULTS AND DISCUSSION

EXTRACTION AND CONCENTRATION

Data from separatory funnel extractions of diphenylamine from reagent water indicated that recoveries of diphenylamine were unacceptably low below the 100 µg/mL conentration level. Recovery data are given in Table 1.

TABLE 1. RECOVERY OF DIPHENYLAMINE FROM WATER USING SEPARATORY FUNNEL TECHNIQUES

Amount Spiked, µg/L	Amount Recovered, µg/L
5	0.4 ± 0.1 (a)
10	2.7 ± 0.3 (b)
50	29 ± 2.6 (b)
100	94 ± 7.7 (b)
500	390 ± 42 (b)

(a) Average of seven extractions; second figure is relative standard deviation.
(b) Average of two extractions; second figure is relative range.

Use of continuous extractors improved recoveries of diphenylamine at lower concentration levels as demonstrated by the recovery data in Table 2.

TABLE 2. RECOVERY OF DIPHEYLAMINE FROM WATER USING CONTINUOUS EXTRACTION TECHNIQUES

Amount Spiked, µg/L	Amount Recovered, µg/L
5	3.6 ± 0.5 (a)
10	8.6 ± 1.2 (b)
50	49 ± 1.7 (b)
100	95 ± 1.9 (b)
500	500 ± 11 (b)
1000	950 ± 8.0 (b)

(a) Average of seven extractions; second figure is relative standard deviation.
(b) Average of two extractions; second figure is relative range.

Use of continuous extractors as opposed to separatory funnels for extraction of diphenylamine from water improved recoveries. For this reason, continuous extractors were used for all diphenylamine method validation studies.

CLEANUP

Diphenylamine eluted from deactivated silica gel in fraction 3 (six percent ethyl ether in petroleum ether). Recoveries of 10 and 100 µg of diphenylamine were 108 and 102 percent, respectively.

CHROMATOGRAPHY

Both the 3% SP-2250 and 3% SP-1000 columns were satisfactory for the GC determination of diphenylamine. The 3% SP-2250 column, however, gave slightly better peak shape and was chosen as the primary column. The following conditions were used for the columns:

Column:	1.8m x 2mm ID 3% SP-2250 on 100/120 mesh Supelcoport or 1.8 m x 2mm ID 3% SP-1000 on 100/120 mesh Supelcoport
Detector:	Alkali flame
Injector Temperature:	280°C
Detector Temperature:	300°C
Oven Temperature:	80°C for 4 minutes; programmed from 80°C to 300°C at 8 C/minute; held at 300°C for 4 minutes (SP-2250 column) 80°C for 4 minutes; programmed from 80°C to 250°C at 8°C/minute; held 250°C for 4 minutes (SP-1000 column).
Carrier Gas:	Helium at 30 mL/minute

Chromatograms obtained under these conditions are shown in Figures 1 and 2.

VALIDATION STUDIES

Recovery of diphenylamine from distilled water at the 5 µg/L level was 3.6 ± 0.5 µg/L. This figure is an average of seven replicate analyses. The MDL in distilled water was calculated to be 1.6 µg/L. Recoveries of diphenylamine from distilled water at the 10, 50, 100, 500, and 1000 µg/L levels were 8.6 ± 1.2, 49 ± 1.7, 95 ± 1.9, 500 ± 11 and 950 ± 8.0 µg/L, respectively. These data were the averages of duplicate analyses. The resultant analytical curve is shown in Figure 3.

Recoveries of diphenylamine from Columbus POTW secondary effluent at the 5 and 50 µg/L levels were 120 ± 25 percent and 89 ± 11 percent, respectively. These data were the averages of seven replicate analyses.

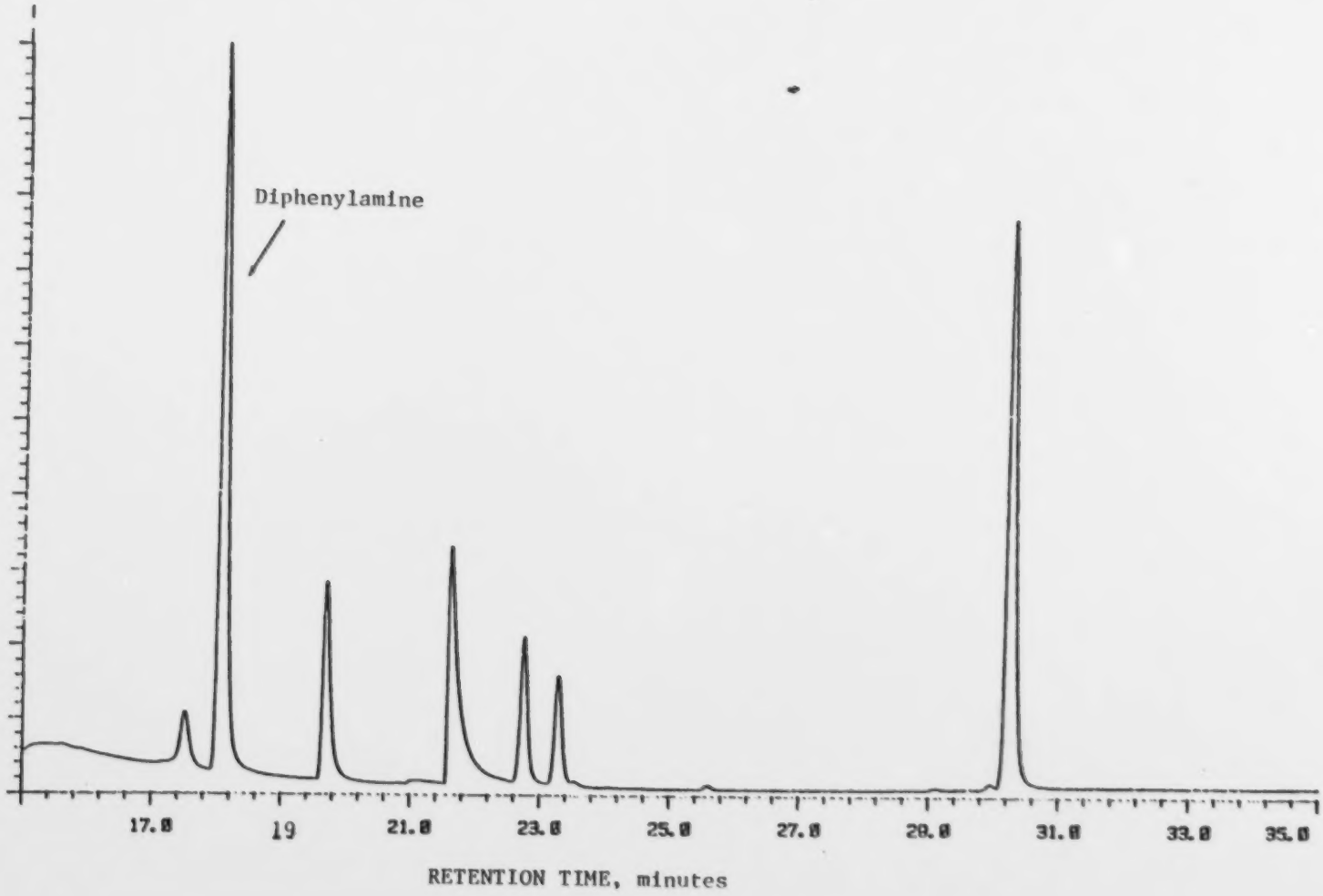


Figure 1. GC-AFD Chromatogram of 100 ng of Diphenylamine (Column 1).

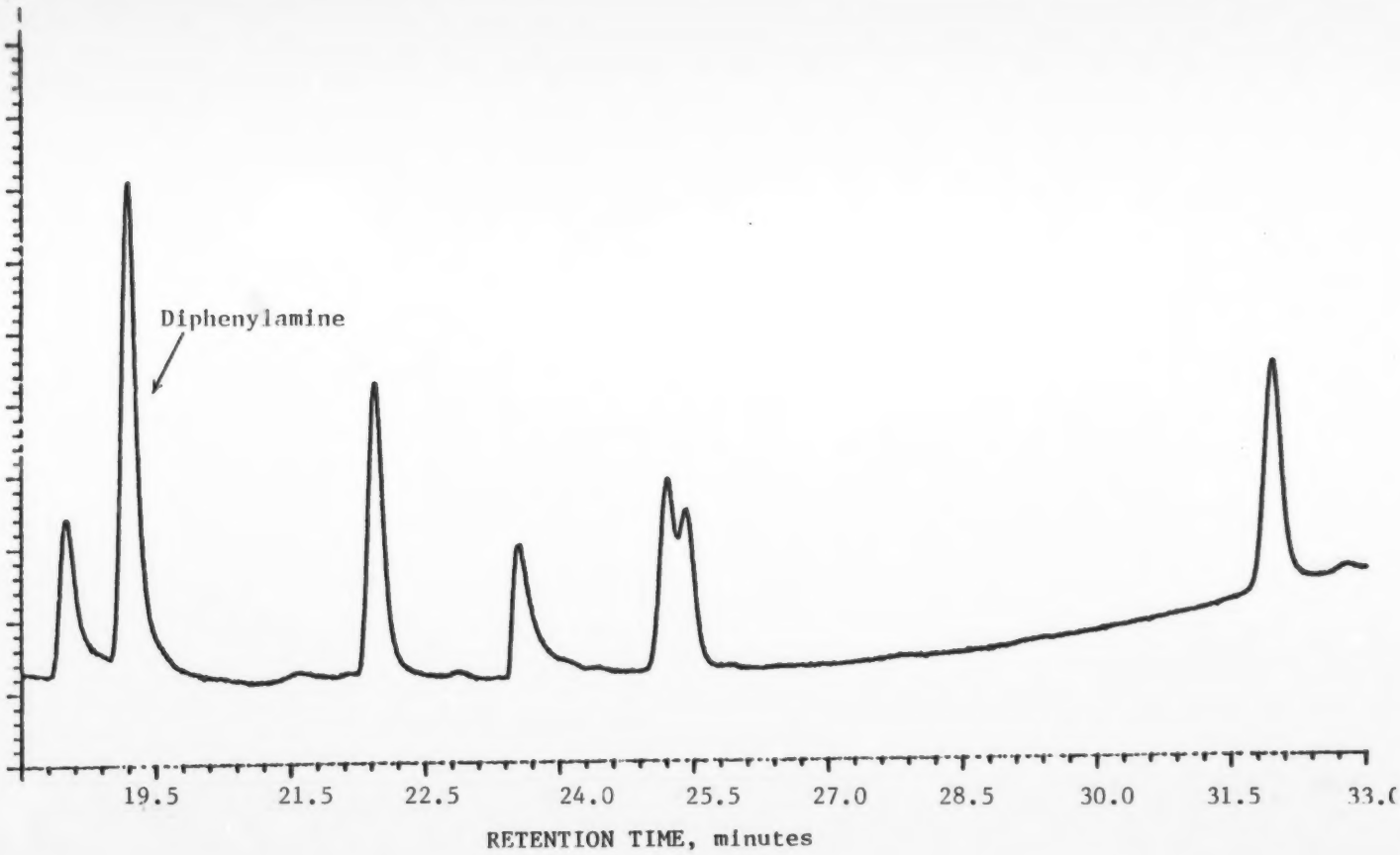


Figure 2. GC-FID Chromatogram of 200 ng of Diphenylamine (Column 2).

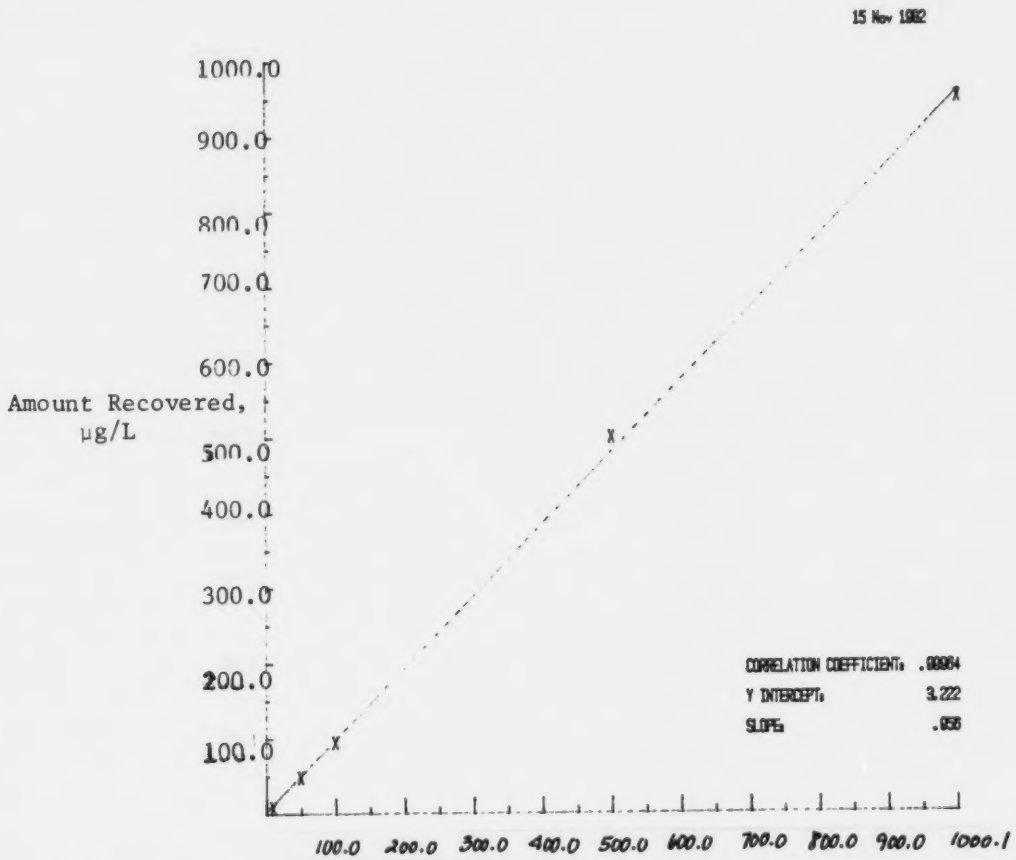


Figure 3. Analytical Curve for Diphenylamine

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METHOD 620 DETERMINATION OF DIPHENYLAMINE IN
MUNICIPAL AND INDUSTRIAL WASTEWATERS
BY GAS CHROMATOGRAPHY

1. Scope and Application

1.1 This method covers the determination of diphenylamine CAS No. 122-39-4.

1.2 This is a gas chromatographic (GC) method applicable to the determination of diphenylamine in municipal and industrial discharges.

1.3 The method detection limit (MDL, defined in Section 15) for diphenylamine is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are similar to those of other 600 series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.

1.5 This method is restrictedto use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate

the ability to generate acceptable results with this method using the procedure described in Section 8.2.

1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC-MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

2.1 A measured volume of sample, approximately 1 liter, is solvent extracted with methylene chloride using a continuous extractor. The methylene chloride extract is dried and concentrated to 5.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector (AFD) gas chromatography.[1]

2.2 This method provides an optional silica gel column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the

analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Thermally stable materials such as PCBs might not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity of carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[3-5] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Aluminum foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the

sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Continuous extractor - 2000-mL, available from Paxton Woods Glass Shop, Cincinnati, Ohio or equivalent.

5.2.2 Drying Column - Chromatographic column 400 mm long x 10 mm ID.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with 250 ml reservoir at the top and Teflon stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 25-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials - Amber glass, 10 to 15 mL capacity with Teflon lined screw-cap.

5.2.9 Volumetric flask - 5-mL with glass stopper.

5.3 Boiling chips - approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.

5.4 Water bath - Heated, capable of temperature control ±2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 3% SP2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Guidelines for the use of alternate columns are provided in Section 12.1.

5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with 3% SP-1000 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector - Alkali-flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method

performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Methylene chloride, acetone, methanol, petroleum ether, ethyl ether, toluene-distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant Test Strips (available from Scientific Products Co., Catalog No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

6.3 Sodium sulfate (ACS) granular, anhydrous; heated in a muffle furnace at 400°C overnight.

6.4 Silica gel - Davison Grade 923, 100-200 mesh; activated by heating for 24 hours at 150°C.

6.5 6N Sulfuric Acid - Slowly add 16.7 mL of conc. H_2SO_4 (94%) to about 50 mL of reagent water. Dilute to 100 mL with reagent water.

6.6 6N Sodium hydroxide - Dissolve 24.0 grams of sodium hydroxide in 100 mL of reagent water.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume

in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with toluene. One of the external standards should be at a concentration near, but above, the method detection limit. The other

concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested, although carbazole has been used successfully in some instances.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with toluene. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples, or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_S C_{is})/(A_{is} C_S)$$

where:

A_S = Response for the compound to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard in μg/L.

C_S = Concentration of the compound to be measured in μg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_S/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permited certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s

Lower Control Limit (LCL) = R = 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts[6] that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R $\pm$ s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation R and s. Alternately, the analyst must use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.[6]

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery

for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Samples Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[7] should be followed; however, the bottle must

not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. Sample Extraction

10.1 Assemble continuous extraction apparatus by placing 5-10 carborundum chips into the 500-mL round-bottom flask and attaching to the extraction flask.

10.2 Add 400 mL methylene chloride to the extraction flask. Some methylene chloride should displace into the round-bottom flask.

10.3 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the extraction flask and add sufficient distilled water to fill the extraction flask (two liters total volume aqueous phase).

10.4 Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 6 N sodium hydroxide or 6 N sulfuric acid.

10.5 Connect the stirring apparatus to the extraction flask without the frit touching the sample. Heat methylene chloride in round-bottom flask to continuous reflux and continue heating for 30 minutes to one hour until frit is thoroughly wetted with methylene chloride.

10.6 Lower frit until it just touches the sample and start the stirring apparatus rotating.

10.7 Continuously extract sample for 18-24 hours.

10.8 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.9 Pour the extract from the round-bottom flask through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the flask and column with 20 to 30 ml of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.

10.10 Add 1 to 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65^{o}C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches approximately 4 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.11 Remove the Snyder column and flask and adjust the volume of the extract to 5.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than two days, transfer the extract to a screw capped vial with a Teflon-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to toluene and gas chromatographic analysis as described in sections 11.5 and 12 respectively. If the sample requires cleanup, proceed to Section 11.

10.12 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Stir 20 g of silica gel in 100 mL of acetone and 1.2 mL of reagent water for 30 minutes on a stirring plate. Transfer the slurry to a chromatographic column (silica gel may be retained with a plug of glass wool). Wash the column with 20 mL of methylene chloride and then with 30 mL of petroleum ether. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.

11.3 Add the extract from Section 10.11 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 6% ethyl ether in petroleum ether. Discard this fraction.

11.4 Elute the column with 100 mL of 15% ethyl ether in petroleum ether and collect in a KD apparatus.

11.5 Add 2.5 mL of toluene to the fraction. Concentrate the fraction to approximately 4 mL with the water bath at 75-80°C as described in Section 10.10. Transfer the sample to a 5-mL volumetric flask and dilute to 5 mL with toluene. Proceed with gas chromatographic analysis.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the gas chromatographic system daily as described in Section 7.

12.3 If an internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 2 to 5 μL of the sample extract using the solvent flush technique.[8] Record the volume injected to the nearest 0.05-μL, and the resulting peak sizes in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected in nanograms.

V_i = Volume of extract injected in μL.

V_t = Volume of total extract in μL.

V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the compound to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract in μg.

V_o = Volume of water extracted in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC-MS Confirmation

14.1 It is recommended that GC-MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from

35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70-V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. When using a fused silica capillary column, the column outlet should be threaded through the interface to within a few mm of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC-MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.

14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC-MS system must be checked to see that all DFTPP performance criteria are achieved.[9]

14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 nanograms of material be injected into the GC-MS. The criteria below must be met for qualitative confirmation.

14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20-40%.

14.4.2 The retention time of the compound in the sample must be within 30 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC-MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. Method Performance

15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in Table 1 were obtained using reagent water.[1] Similar results were achieved using representative wastewaters.

15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 10 x MDL to 1000 x MDL.

15.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.[1]

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TABLE 1. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention Time (min)		Method Detection Limit (μg/L)
	Column 1	Column 2	
Diphenylamine	18.1	19.3	1.6

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is held at 80°C for 4 minutes, programmed from 80°C to 300°C at 8°C/min and held at 300°C for 4 minutes.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-1000 packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is held at 80°C for 4 minutes, programmed from 80°C to 250°C at 80°C/min, and held at 250°C for 4 minutes.

TABLE 2. SINGLE LABORATORY ACCURACY AND PRECISION(a)

Parameter	Average Percent Recovery	Relative Standard Deviation, %	Spike Level (µg/L)	Number of Analyses	Matrix Type(b)
Diphenylamine	120	25	5.0	7	1
	89	11	50	7	1

(a) Column 1 conditions were used.

(b) 1 = Columbus secondary POTW effluent.

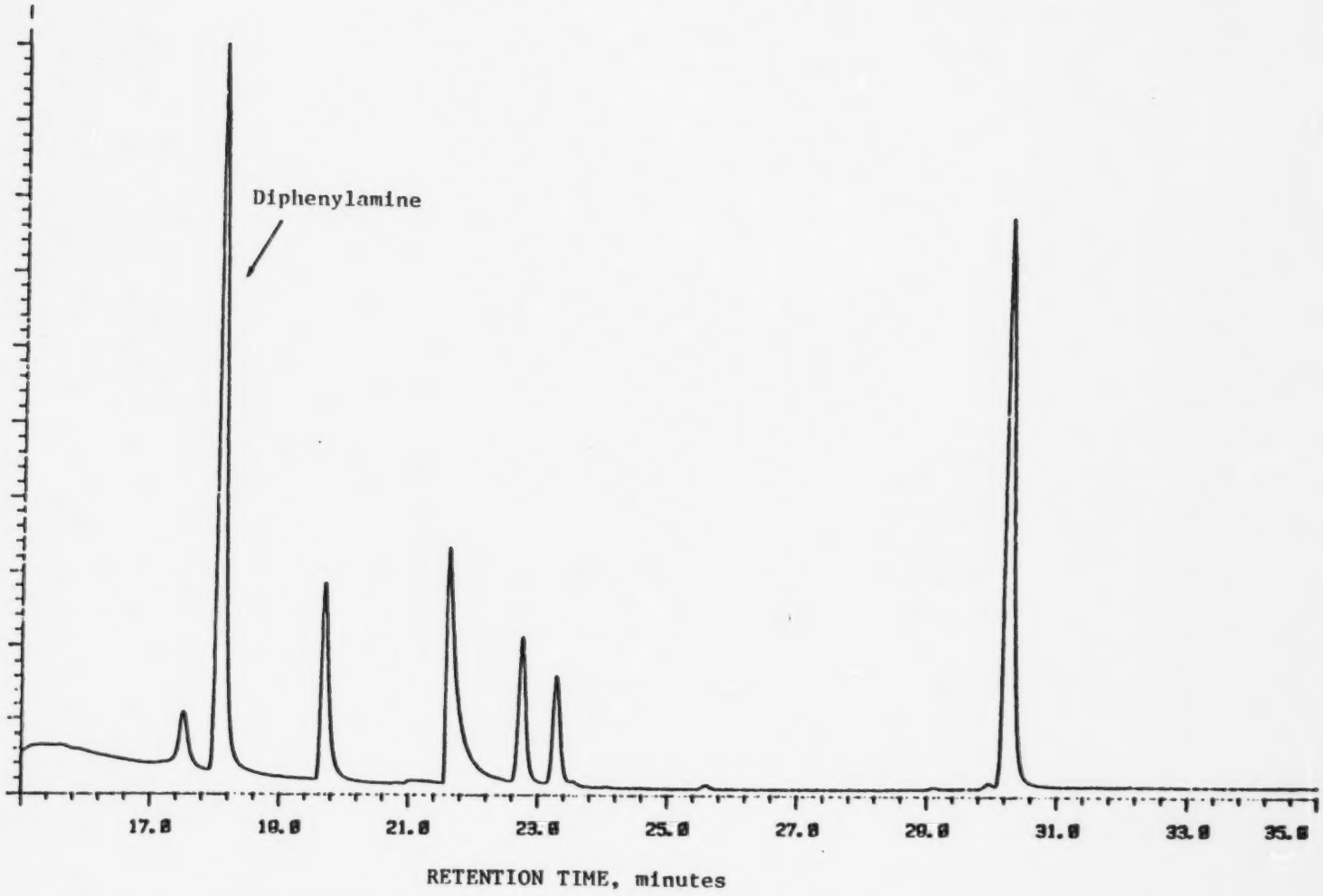

Figure 1. GC-AFD Chromatogram of 100 ng of Diphenylamine (Column 1).

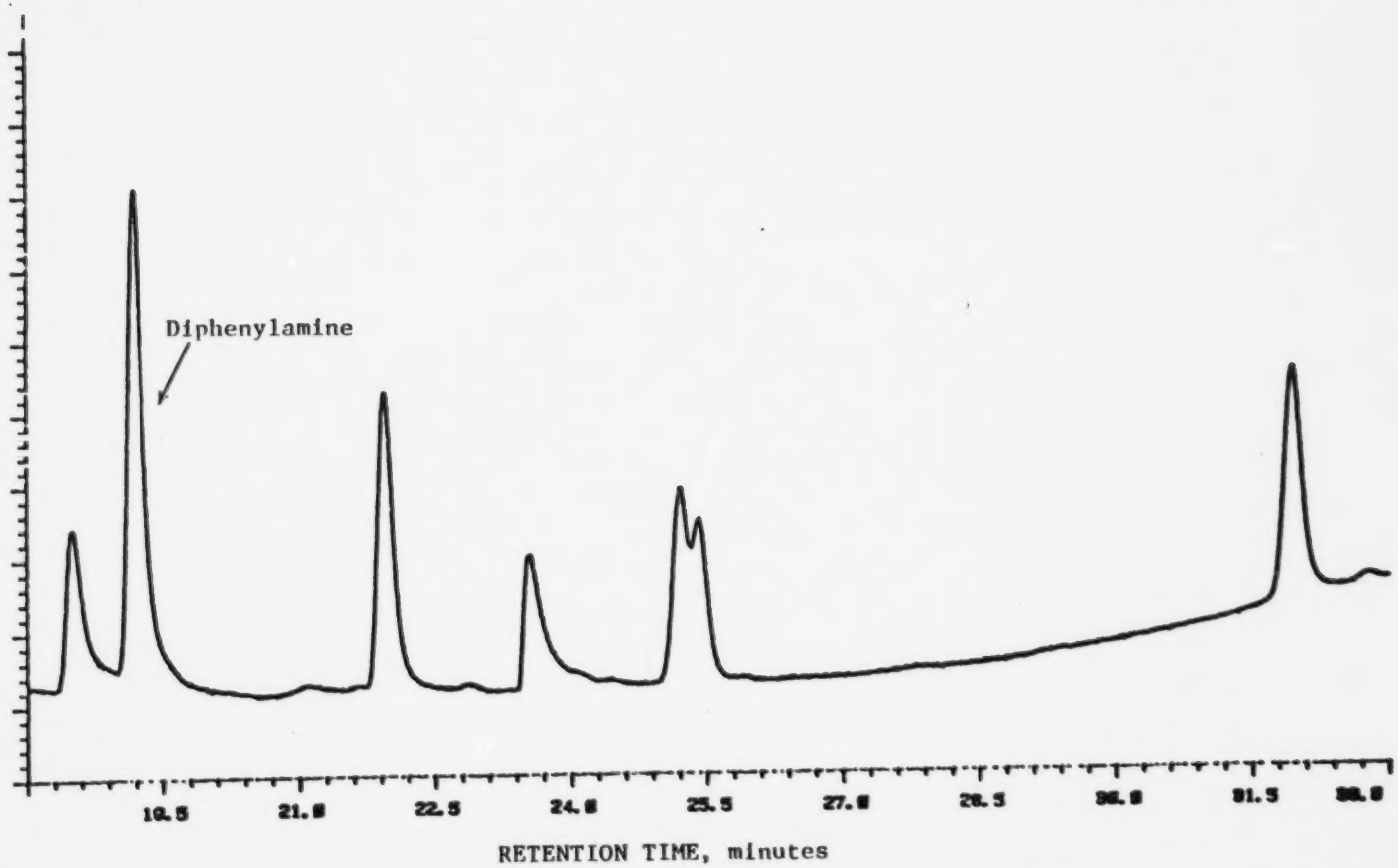

Figure 2. GC-FID Chromatogram of 200 ng of Diphenylamine (Column 2).